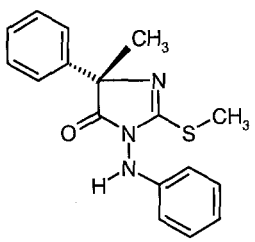
Data Evaluation Report on the phototransformation of fenamidone on soil

PMRA Submission Number {.....} EPA MRID Number 45385832

Data Requirement: PMRA Data Code:
EPA DP Barcode: D275213
OECD Data Point:
EPA Guideline: 161-3

Test material:
Common name: Fenamidone
Chemical name
IUPAC: (+)-(4*S*)-4-Methyl-2-methylthio-4-phenyl-(1H)-1-phenylamino-2-imidazolin-5-one.
CAS name: 4*H*-Imidazol-4-one, 3,5-dihydro-5-methyl-2-(methylthio)-5-phenyl-3-(phenylamino)-, (*S*)-.
CAS No: 161326-34-7.
Synonyms: Reason 500 SC Fungicide.
Methyl-2-methylthio-5-phenyl-3-phenylamino-3,5-dihydro-4H-imidazol-4-one.
(*S*)-1-Anilino-4-methyl-2-methylthio-4-phenylimidazolin-5-one.
(*S*)-5-Methyl-2-methylthio-5-phenyl-3-phenylamino-3,5-dihydroimidazol-4-one.
Imidazol-4-one, 3,5-dihydro-5-methyl-2-(methylthio)-5-phenyl-3-(phenylamino)-, (5*S*)-.
(5*S*)-3,5-Dihydro-5-methyl-2-(methylthio)-5-phenyl-3-(phenylamino)-4*H*-imidazol-4-one.
RPA407213.
SMILES string:

Chemical Structure:



Primary Reviewer: Lynne Binari
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Company Code: [for PMRA]
Active Code: [for PMRA]

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Imidazol-4-one, 3,5-dihydro-5-methyl-2-(methylthio)-5-phenyl-3-(phenylamino)-, (5*S*)-.
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Company Code: [for PMRA]
Active Code: [for PMRA]
Use Site Category: [for PMRA]
EPA PC Code: 046679

CITATION: Burr, C.M. and A.J. McDonald. 1999. [^{14}C]-RPA 407213 soil photolysis. Unpublished study performed by Rhône-Poulenc Agricultural Ltd., Essex, United Kingdom, and sponsored by Aventis CropScience, Research Triangle Park, NC (pp. 1, 2). Laboratory Study Number 10608. Document No. 201428. The study was completed May 27, 1999 (p. 1); a study initiation date was not reported, but the experimental start date was July 12, 1996 (p. 31).

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Use Site Category: [for PMRA]
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CITATION: Burr, C.M. and A.J. McDonald. 1999. [^{14}C]-RPA 407213 soil photolysis. Unpublished study performed by Rhône-Poulenc Agricultural Ltd., Essex, United Kingdom, and sponsored by Aventis CropScience, Research Triangle Park, NC (pp. 1, 2). Laboratory Study Number 10608. Document No. 201428. The study was completed May 27, 1999 (p. 1); a study initiation date was not reported, but the experimental start date was July 12, 1996 (p. 31).

Regulatory Conclusions: This study, conducted with [C-phenyl-U-^{14}C]-labeled fenamidone, is classified acceptable and partially satisfies Subdivision N Guideline §161-3 data requirements. This study plus the soil photolysis study conducted with [N-phenyl-U-^{14}C]-labeled fenamidone (MRID 45385901) fully satisfy Subdivision N Guideline §161-3.

EXECUTIVE SUMMARY

The phototransformation of [C-phenyl-U-^{14}C]-labeled (+)-(4*S*)-4-methyl-2-methylthio-4-phenyl-(1H)-1-phenylamino-2-imidazolin-5-one (fenamidone, RPA407213) was studied on sandy loam soil (pH in water 6.7, organic carbon 1.2%) from Wisconsin at a nominal concentration of 19.9 mg a.i./kg soil for 30 days at 20 ± 1°C and 75% of 0.33 bar moisture. This experiment was conducted in accordance with USEPA Subdivision N Guideline §161-3 and in compliance with OECD GLP in the Testing of Chemicals (Paris, 1982), the U.K. Principles of GLP of the U.K. Compliance Programme (1989) and the U.K. GLP Regulations (1997, No. 654 Health & Safety). The treated samples were irradiated under a 13.3-hour daylight/10.7-hour darkness photoperiod using a UV-filtered xenon lamp (290-800 nm, average light intensity 311 W/m^2); it was calculated that 13.3 hours of xenon lamp irradiation was equivalent to 1 day of clear midday summer sunlight at 50°N latitude. Each irradiated test system consisted of treated soil contained in an open quartz dish (2.6-cm dia.) incubated within a double-walled glass vessel (4-cm dia.) sealed with a quartz disc and equipped with inlet/out ports to allow for collection of CO_2 and organic volatiles at each sampling interval. Duplicate treated dark control soils were similarly incubated in a temperature-controlled room. Irradiated and dark control soils were taken after 0, 2, 5, 9, 15, 21 and 30 days. All soil samples were sequentially extracted with acetonitrile and acetonitrile:water (1:1, v:v), with all soil samples after day 0 also Soxhlet-extracted with acetonitrile:water (1:1, v:v) followed by deionized water. Soil extracts were analysed by reverse-phase HPLC and normal-phase one-dimensional TLC; identifications of fenamidone and transformation products were based on comparative HPLC retention times and TLC R_f values with unlabeled reference standards. Identifications of [^{14}C]compounds were confirmed using LC/MS with electrospray ionization (ESP) and LC/MS-ESP with multiple reaction monitoring (MRM).

During the 30-day study, mean (n = 2) material balances decreased from an initial 101.6 ± 1.34% of the applied radioactivity to 91.9 ± 0.41% in irradiated soil and 87.9 ± 2.54% in dark control soil at 21 days posttreatment and were 94.7 ± 0.6% and 95.2 ± 2.22% at 30 days, respectively. Irradiation did not significantly affect the rate of transformation of [C-phenyl-^{14}C]fenamidone or the transformation products formed when incubated on sandy loam soil at 20 ± 1°C and 75% of 0.33 bar moisture. [^{14}C]Fenamidone decreased in dark control soil extracts from 99.96 ± 1.30% of the applied at day 0 to 45.88 ± 0.82% at 5 days, 22.67 ± 0.88% at 15 days and was 10.59 ± 2.09% at 30 days. In irradiated soil extracts, [^{14}C]fenamidone decreased to 49.03 ± 0.84% at 9 days and was 26.01 ± 1.53% at 30 days. The slower rate of transformation of [^{14}C]fenamidone on the irradiated soil may have been the result of difficulty in maintaining the soil moisture content at a constant level. The two major transformation products identified in irradiated and dark control soil extracts

were RPA408056 (5-methyl-2-methylthio-5-phenyl-3,5-dihydroimidazol-4-one) and RPA717879 (5-methyl-5-phenylimidazolidine-2,4-dione). RPA408056 was detected at maximums of 20.41% (9 days) and 17.78% (30 days) of the applied radioactivity in the dark control and irradiated soil extracts, respectively. RPA717879 was detected at maximums of 11.21% (21 days) and 12.44% (30 days), respectively. Minor transformation products in the soil extracts included RPA409445 (3-(4-aminophenylamino)-5-methyl-5-phenylimidazolidine-2,4-dione), RPA405862 (5-methyl-5-phenyl-3-phenylaminoimidazolidine-2,4-dione), RPA406012 (5-methyl-2-methylthio-3-(4-nitrophenylamino)-5-phenyl-3,5-dihydroimidazol-4-one) and RPA410914 (5-methyl-2-methylthio-3-(2-nitrophenylamino)-5-phenyl-3,5-dihydroimidazol-4-one; dark soil extracts only), plus up to twenty-one unidentified [^{14}C]compounds each detected at <4% of the applied radioactivity. At the end of the study, volatilized $^{14}CO_2$ totaled 8.13 ± 2.0% of the applied for dark control soils and 2.25 ± 0.34% for irradiated soils; organic [^{14}C]volatiles were ≤0.03% of the applied.

Extractable [^{14}C]residues decreased from 101.15 ± 1.33% of the applied at day 0 to 65.34-73.34% at 21-30 days in dark control soil and 76.61-86.25% at 15-30 days in irradiated soil; over the same intervals nonextractable [^{14}C]residues in increased from 0.49 ± 0.0% of the applied to 13.51-16.35% in dark control soil and 8.36-11.47% in irradiated soil. For nonextractable [^{14}C]residues in 9- and 30-day dark control and 15- and 30-day irradiated soil samples, 2.67-4.14% of the applied was associated with the fulvic acid, 2.80-4.81% with humic acid and 4.64-7.40% with humins.

Half-life values of [C-phenyl-U-^{14}C]fenamidone, based on first-order kinetics and linear regression, were 9.5 days (r^2 = 0.927) on dark control soil and 16.4 days (r^2 = 0.899) on irradiated soil based on the continuous irradiation conditions used in this study. Registrant-calculated nonlinear (two compartment decay) DT_{50} values were 3.6 and 9.2 days on dark control and irradiated soil, respectively, with respective DT_{90} values of 25.9 and 49.9 days.

Since the rate of degradation was much faster in the dark control than the irradiated samples, the **phototransformation half-life** could not be determined.

The transformation of fenamidone on soil was attributed solely to metabolic processes, since no unique transformation products resulted from irradiation. A transformation pathway for fenamidone on soil proposed by the registrant included fenamidone degrading to 5-methyl-2-methylthio-5-phenyl-3,5-dihydroimidazol-4-one (RPA408056) via loss of the aniline ring, with further degradation to 5-methyl-5-phenylimidazolidine-2,4-dione (RPA717879) via hydrolysis to release the methylthio group and eventual mineralization to CO_2.

Results Synopsis:

Soil type:	Sandy loam.
Source of irradiation:	Artificial xenon lamp.
Half-life value for dark:	9.5 days (r^2 = 0.927).
Half-life value for irradiated:	16.4 days (r^2 = 0.899).

Major transformation products: 5-Methyl-2-methylthio-5-phenyl-3,5-dihydroimidazol-4-one (RPA408056).
5-Methyl-5-phenylimidazolidine-2,4-dione (RPA717879).

Minor transformation products: 3-(4-Aminophenylamino)-5-methyl-5-phenylimidazolidine-2,4-dione (RPA409445).
5-methyl-5-phenyl-3-phenylaminoimidazolidine-2,4-dione (RPA405862).
5-Methyl-2-methylthio-3-(4-nitrophenylamino)-5-phenyl-3,5-dihydroimidazol-4-one (RPA406012).
5-Methyl-2-methylthio-3-(2-nitrophenylamino)-5-phenyl-3,5-dihydroimidazol-4-one (RPA410914).
Twenty-one unidentified [^{14}C]compounds.
CO_2.

Study Acceptability:

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted in accordance with USEPA Subdivision N Guideline §161-3 and EU Commission Directive 95/36/EC of July 1995 amending Council Directive 91/414/EEC, Section 7.1.1.1.2 (p. 14). No significant deviations were noted.

COMPLIANCE: This study was conducted in compliance with OECD GLP in the Testing of Chemicals (Paris, 1982), the U.K. Principles of GLP of the U.K. Compliance Programme (1989) and the U.K. GLP Regulations (1997, No. 654 Health & Safety; p. 3). Signed and dated GLP, Data Confidentiality, study Authentication, study Certification and Quality Assurance statements were provided (pp. 2-5, 31, 32).

A. MATERIALS:

1. Test Material: [C-Phenyl-U-^{14}C]RPA407213

Chemical Structure:

Description: Not provided.

Purity:

[C-Phenyl-U-^{14}C]-labeled: Radiochemical purity: >98% (pp. 14, 15; Appendix 1, p. 103).
Batch No.: CFQ9085.
Optical purity: 99.3% (Appendix 1, p. 104).
Initial specific activity: 38 mCi/mM (1.41 GBq/mM).

Final specific activity following dilution with unlabeled fenamidone: 19 mCi/mM (695 MBq/mM; Appendix 6, p. 120).

Unlabeled: Purity 99.6% (p. 15).
Batch No. MCD1905.

Storage conditions of test chemical: Not specified.

Table 1: Physico-chemical properties of fenamidone.

Parameter	Details	Comments
Solubility:	7.8 mg/L in water at 20°C. 86.1 g/L in acetonitrile at 20°C.	Data obtained from p. 10, MRID 45385831.
Vapor pressure/volatility:	Not reported.	
UV absorption:	<300 nm	In methanol:pH 7 buffer solution (90:10, v:v; p. 57, MRID 45385830).
pK_a:	Not reported.	
K_{ow}	Not reported.	
Stability at room temperature:	Not reported.	

2. Soil Characteristics:

Table 2: Description of soil collection and storage.

Description	Nisse
Geographic location:	Hill Top Farm, Iola, Wisconsin, U.S.A.
Collection date:	June 28, 1996.
Storage conditions:	4°C.
Soil preparation:	2 mm sieved.

Data obtained from p. 15 in the study report.

Table 3: Properties of the soils.

Property	Nisse
Soil texture:	Sandy loam.
% sand (50-2000 µm):	64.17.
% silt (2-50 µm):	29.11.
% clay (<2 µm):	6.72.
pH:	6.7 in water. 6.0 in 1 M KCl. 5.9 in 0.01 M $CaCl_2$.
Organic carbon (%): Organic matter (%, calculated):	1.2. 2.1.
CEC (meq/100 g):	6.5.
Moisture at 1/3 atm (%):	20.66.
Bulk density (g/cm^3):	Not provided.
Microbial biomass (µg C/g soil):	183; fumigation-extraction method.
Soil Taxonomic classification:	Not provided.
Soil Mapping Unit (for EPA):	Not provided.

Data obtained from p. 34 in the study report.

3. Details of light source:

Table 3: Artificial light source.

Property	Details
Type of lamp used:	Xenon lamp, Heraeus Hanau Suntest.
Emission wavelength spectrum:	290-800 nm.
Light intensity:	Average 311.4 W/m^2, measured with a Heraeus Radialux global sensor (wavelength range not specified) at same distance as soil surface.
Filters used:	UV filter eliminated radiation <290 nm.
Relationship to natural sunlight:	13.3 hours of xenon lamp irradiation was equal to 1 day of clear midday summer sunlight at 50°N latitude.

Data obtained from p. 16; Appendix 2, pp. 105-106 in the study report.

B. EXPERIMENTAL CONDITIONS:

1. Preliminary experiments: None.

2. Experimental conditions:

Table 4: Experimental design.

Parameter			Details
Duration of the test:			30 days.
Condition of soil:	Air dried/fresh:		Fresh.
	Sterile/non-sterile:		Non-sterile.
Soil (g/replicate):			4 g dry wt.
Test concentration:		Nominal:	19.9 mg a.i./kg soil; 22.5 kg a.i./ha.
		Actual:	19.4 mg a.i./kg soil; 21.9 kg a.i./ha.
Dark controls used (Yes/No): Method to maintain darkness:			Yes. Samples were incubated in darkness in a temperature-controlled room.
Replications	Dark controls:		Duplicate.
	Irradiated:		Duplicate.
Identity and concentration of co-solvent:			*Acetonitrile, 100%.*
Test material application.	Volume of test solution used/treatment:		0.025 mL of 3.15 mg a.i./mL test solution.
	Application method:		Applied drop-wise to soil surface. Soil was not mixed following application.
	Is the co-solvent evaporated?		No.
Test apparatus (Type/material/ volume):			An open quartz dish (2.6 cm diameter), containing treated soil (depth ca. 1 cm), was incubated within a double-walled, glass vessel (4 cm diameter, 13.1 cm height) equipped with inlet/outlet ports and sealed with a quartz disc.
Details of traps for CO_2 and organic volatiles, if any:			Humidified, CO_2-free air was forced (flow rate not specified) through each vessel, then sequentially through traps containing ethylene glycol (one trap) and 2 M KOH (two traps).
If no traps were used, is the system closed/open?			Volatiles traps were used.
Any indication of the test material adsorbing to the walls of the test apparatus?			Not indicated.

Parameter			Details
Experimental conditions.	Temperature (°C):	Dark controls:	Temperature controlled room maintained at 20 ± 1°C.
		Irradiated:	Soil surface maintained at 20 ± 1°C via a water:ethylene glycol coolant circulated through the double-wall of each vessel with a flow-heater/cooler. The soil surface temperature was monitored with a temperature probe connected to the flow-heater/cooler.
	Moisture content: Moisture maintenance method:		75% of 0.33 bar. Samples weighed daily; water added, as needed, equivalent to weight lost.
	Duration of light/darkness:		13.3-hour light/10.7-hour dark cycle.
	Distance from light source to soil surface:		29.5 cm.
Other details, if any:			None.

Data obtained from pp. 15-17; Figure 1, p. 51; Appendix 6, pp. 120-121 in the study report.

3. Supplementary experiments: None.

4. Sampling:

Table 5: Sampling details.

Parameters	Details
Sampling intervals for soil:	0, 2, 5, 9, 15, 21 and 30 days.
Sampling method:	Duplicate day 0 samples. Duplicate irradiated and dark control samples at all other intervals.
Method of collection of volatile compounds, if any:	Trapping solutions were collected and replaced at each sampling interval after day 0.
Sampling intervals/times for: Sterility check: Moisture content:	 Sterile controls were not used. At each sampling interval.
Sample storage before analysis:	Extraction procedures initiated upon sample collection.
Other observations, if any:	None

Data obtained from p. 17; Table 2, p. 35 of the study report.

C. ANALYTICAL METHODS:

Extraction/clean up/concentration methods: Each soil sample was extracted with acetonitrile (25 mL x 2) followed by acetonitrile:water (1:1, v:v; 25 mL) using a wrist action shaker for 15 minutes per extraction (p. 17). All extracted soil samples, except day 0, were further Soxhlet-extracted with

acetonitrile:water (1:1, v:v; 50 mL) followed by deionized water (50 mL) for 20 hours per extraction, with a final water extraction (15 mL x 2) using a wrist action shaker for 5 minutes per extraction (p. 18). All extracts were separated from the soil by centrifugation with like extracts combined. The day 0 acetonitrile extract was analyzed directly.

For all remaining soil extracts, the acetonitrile and acetonitrile:water extracts were combined, an aliquot (25 mL) was concentrated to <0.5 mL by rotary evaporation (40°C), then the concentrated sample was redissolved in acetonitrile:water (1:1, v:v; 1.5 mL) and brought to final volume (5 mL) with acetonitrile. An aliquot (20 mL) of the Soxhlet acetonitrile:water extract was similarly concentrated prior to analysis (p. 19). An aliquot (50 mL) of the Soxhlet water extract was freeze-dried, then the residues were redissolved in water (1 mL), diluted with acetonitrile:water (2 mL) and brought to volume (5 mL) with acetonitrile. Triplicate aliquots (0.25-1.0 mL) of the soil extracts were analyzed for total radioactivity by LSC (Appendix 6, pp. 122-143).

Nonextractable residue determination: To characterize unextractable [^{14}C]residues in the soil, selected previously extracted soil samples were further extracted with 0.1 N sodium hydroxide for 24 hours using a wrist action shaker (p. 19). The extract was separated from soil by centrifugation, acidified with concentrated hydrochloric acid to pH 1, and the resulting precipitate (humic acid) was removed by centrifugation. The remaining extract (fulvic acid) was analyzed by LSC. The humic acid precipitate was redissolved in 0.1 M sodium hydroxide and analyzed by LSC. [^{14}C]Residues remaining in the extracted soil (humin) were determined by subtraction. Extracted soil samples plus respective cellulose thimbles (from Soxhlet extractions) were air-dried, homogenized using a Labtechnics LM1-P mill, then triplicate aliquots were analyzed for total radioactivity by LSC following combustion (p. 19).

Volatile residue determination: Aliquots (1 mL) of each trapping solution were analyzed for total radioactivity by LSC (Appendix 6, pp. 129, 130, 137, 138, 144, 145). To quantify $^{14}CO_2$, aliquots (10 mL) of selected potassium hydroxide trapping solutions were reacted with 1 M sodium carbonate (1 mL) and 1 M barium chloride (4 mL) to precipitate the $^{14}CO_2$ as [^{14}C]barium carbonate (p. 20). Solution and barium carbonate precipitate were separated by centrifugation, then the solution was analyzed by LSC.

Total ^{14}C measurement: Total ^{14}C residues were determined by summing the concentration of residues measured in the soil extracts, extracted soil, and volatile trapping solutions.

Derivatization method, if used: A derivatization method was not employed.

Identification and quantification of parent compound and transformation products: Aliquots of the day 0 soil extracts were analyzed by reverse-phase HPLC Method 1 under the following conditions: Hichrom Kromasil KR100-5C8 column (4.6 x 250 mm, particle size not specified), gradient mobile phase combining (A) water:acetonitrile (80:20, v:v) and (B) water:acetonitrile (50:50, v:v) [percent A:B at 0 min. 100:0 (v:v), 8 min. 100:0, 15 min. 0:100, 35 min. 0:100, 36 min. 100:0, 40 min. 100:0], injection volume and flow rate not specified, UV (230 nm) and radioactivity

detection (pp. 20, 21; Figure 8, p. 55; Figure 10, p. 57). Aliquots of soil extracts after day 0 were analyzed by reverse-phase HPLC Method 2 under the following conditions: Hichrom Kromasil KR100-5C1 column (4.6 x 250 mm, particle size not specified), gradient mobile phase combining (A) water:acetonitrile (80:20, v:v), (B) water:acetonitrile (50:50, v:v) and (C) acetonitrile [percent A:B:C at 0 min. 100:0:0 (v:v), 10 min. 100:0:0, 15 min. 0:100:0, 30 min. 0:100:0, 55 min. 0:0:100, 58 min. 0:0:100, 60 min. 100:0:0, 65 min. 100:0:0], and all other conditions as described for Method 1 (p. 21; Figure 9, p. 56, Figures 11-26, pp. 58-73). Unlabeled reference standards (Table 16, pp. 48-50) were chromatographed with the extracts and identifications of [^{14}C]fenamidone in the soil extracts were based on comparative retention times.

Soil extracts were also analyzed by normal-phase one-dimensional TLC conducted on silica gel plates developed with ethyl acetate:hexane (40:60, v:v; p. 20; Figures 27-34, pp. 74-81). Unlabeled RPA407213 was co-chromatographed with the soil extracts. Following development, distribution of radioactivity was determined using an Ambis Radioanalytical Imaging System. Unlabeled reference compounds were detected under UV light (254 nm) and identifications of [^{14}C]fenamidone and transformation products in the soil extracts were based on comparative R_f values.

Identifications of parent fenamidone in selected soil extracts were confirmed by full scan LC/MS with electrospray ionization (ESP; pp. 22-24; Figures 35-46, pp. 82-93). Comparisons were made to an unlabeled reference standard of parent fenamidone (RPA407213). LC conditions were as described for Method 2 above, except solvent B was water:acetonitrile (60:40, v:v). MS conditions were as follows: VG Quattro I Triple Quadripole MS, bath gas nitrogen at 300 L/hour, nebulizer gas nitrogen at 20 L/hour, source temperature 140°C, capillary voltage 3.0-4.0 kV, HV lens 0.50 kV, cone voltage 25 V, multiplier 650 V, positive ion scan range 150-700 a.m.u. at 2 sec./scan cycle, negative ion scan range 100-800 a.m.u. at 2 sec./scan cycle.

Identification and quantification of transformation products: Transformation products were isolated and quantified by HPLC, TLC, and MS as described for the parent compound and identified by comparison to reference standards (Table 16, pp. 48-50). Identification of [^{14}C]RPA717879 was also confirmed by LC/MS-ESP with multiple reaction monitoring (MRM; Figures 47-52, pp. 94-99). LC conditions were as follows: Hichrom Kromasil KR100-5C1 column (4.6 x 250 mm, particle size not specified), isocratic mobile phase of acetonitrile:water (60:40, v:v), injection volume 100 µL, flow rate 1.0 mL/minute with 20:1 split on column ca. 0.05 mL/minute into ESP ion source, UV and radioactivity detection (pp. 20-22). MS conditions were as follows: Micromass Quattro Triple Quadripole MS, drying gas nitrogen at 1,100 L/hour, nebulizer gas nitrogen at 99 L/hour, collision gas argon at 3.1 x 10^{-4} mBar, source temperature 150°C, capillary voltage 3.5 kV, simmer lens 2.0 V, cone voltage 32-37 V, collision energy 40-50 eV, multiplier 650 V, negative ion scan range 40-250 a.m.u. at 1 sec./scan cycle and negative ion MRM scan range 189-42 at a 1 second cycle (pp. 23-24).

Detection limits (LOD, LOQ) for the parent compound: Limits of detection for LSC, HPLC and TLC analyses were not reported.

Detection limits (LOD, LOQ) for the transformation products: Limits of detection for LSC, HPLC and TLC analyses were not reported.

II. RESULTS AND DISCUSSION

A. TEST CONDITIONS: Moisture, temperature and other environmental conditions were reported to have been maintained throughout the study. No supporting records were provided.

B. MATERIAL BALANCE: Mean (n = 2) total recoveries of radiolabeled material decreased from an initial 101.6 ± 1.34% (range 100.3-102.98%) of the applied to 91.9 ± 0.41% (91.52-92.33%) in irradiated soil and to 87.9 ± 2.54% (85.4-90.48%) in dark control soil at 21 days posttreatment and were 94.7 ± 0.6% (94.13-95.33%) and 95.2 ± 2.22% (93.0-97.43%) at 30 days, respectively (Tables 3-4, pp. 36, 37).

Table 6: Phototransformation of [C-phenyl-^{14}C]fenamidone on sandy loam soil, expressed as percentage of applied radioactivity (mean ± s.d., n = 2).

Compound and/or code		Sampling times (days)						
		0	2	5	9	15	21	30
Fenamidone (RPA407213)	irradiated	99.96 ± 1.30	73.11 ± 0.52	64.90 ± 1.98	49.03 ± 0.84	37.07± 6.79	33.23 ± 1.38	26.01 ± 1.53
	dark		62.93 ± 1.30	45.88 ± 0.82	30.48 ± 2.53	22.67± 0.88	13.51 ± 0.79	10.59 ± 2.09
RPA408056	irradiated	0.00	4.61 ± 0.88	10.16 ± 0.14	15.50 ± 2.52	12.48 ± 0.35	15.10 ± 0.31	16.83 ± 0.95
	dark		2.88 ± 2.88	11.16 ± 1.56	19.50 ± 0.90	12.15 ± 0.10	9.03 ± 0.01	11.75 ± 1.22
RPA717879	irradiated	0.00	0.97 ± 0.97	0.98 ± 0.02	4.67 ± 1.59	9.35 ± 0.77	9.62 ± 0.02	11.80 ± 0.64
	dark		3.16 ± 3.17	2.35 ± 1.1	4.60 ± 0.35	7.25 ± 0.48	9.98 ± 1.23	9.55 ± 0.13
RPA409445	irradiated	0.00	0.00	0.69 ± 0.44	2.49 ± 0.45	2.41 ± 0.06	1.87 ± 0.17	2.73 ± 0.45
	dark		0.67 ± 0.08	1.32 ± 0.14	2.38 ± 0.2	2.14 ± 0.12	0.68 ± 0.68	1.28 ± 0.37
RPA405862	irradiated	0.00	0.59 ± 0.11	1.04 ± 0.12	1.75 ± 0.31	1.78 ± 0.22	1.53 ± 0.02	1.9 ± 0.44
	dark		0.7 ± 0.02	0.81 ± 0.02	0.61 ± 0.1	1.14 ± 0.35	2.40 ± 0.16	1.66 ± 0.01
RPA406012	irradiated	0.00	0.53 ± 0.01	0.72 ± 0.17	0.92 ± 0.12	0.69 ± 0.11	0.48 ± 0.09	0.53 ± 0.03
	dark		0.72 ± 0.05	0.68 ± 0.05	0.73 ± 0.05	0.68 ± 0.07	0.16 ± 0.17	0.46 ± 0.24
RPA410914	irradiated	0.00	ND	ND	ND	ND	ND	ND
	dark		0.00	0.00	0.00	0.00	0.22 ± 0.23	0.28 ± 0.28
Unidentified radioactivity (RT <33 min.)[1]	irradiated	<LOD	0.85 ± 0.20	1.63 ± 0.38	3.16 ± 0.41	2.58 ± 0.07	3.40 ± 0.04	4.99 ± 0.36
	dark		0.54 ± 0.24	1.37 ± 0.08	2.88 ± 0.46	3.64 ± 0.42	5.04 ± 0.47	7.85 ± 1.61
Unidentified radioactivity (RT >39 min.)[2]	irradiated	<LOD	6.37 ± 0.02	8.53 ± 0.44	11.11 ± 1.08	15.06 ± 1.07	15.21 ± 0.82	17.54 ± 0.76
	dark		11.67 ± 0.83	19.12 ± 0.73	24.56 ± 1.19	27.05 ± 0.07	26.06 ± 1.4	28.74 ± 0.22
Total extractable [^{14}C]residues	irradiated	101.15 ± 1.33	92.34 ± 0.41	91.11 ± 1.19	88.62 ± 0.13	81.43 ± 4.82	80.46 ± 0.36	82.33 ± 0.12
	dark		91.36 ± 0.35	86.68 ± 0.64	85.76 ± 0.3	76.71 ± 0.58	67.07 ± 1.73	72.14 ± 1.19

Compound and/or code		Sampling times (days)						
		0	2	5	9	15	21	30
Unextractable [^{14}C]residues	irradiated	0.49 ± 0.0	3.26 ± 0.19	4.90 ± 0.39	7.10 ± 0.49	9.92 ± 1.56	9.83 ± 0.78	10.15 ± 0.38
	dark		5.85 ± 0.63	7.79 ± 0.68	10.67 ± 0.41	12.57 ± 0.04	14.35 ± 0.06	14.93 ± 1.42
CO_2 and other volatiles[3]	irradiated	NA	0.16 ± 0.02	0.35 ± 0.05	0.81 ± 0.09	1.15 ± 0.06	1.64 ± 0.01	2.25 ± 0.34
	dark		0.34 ± 0.13	0.50 ± 0.03	1.41 ± 0.07	3.50 ± 0.37	6.53 ± 0.86	8.13 ± 2.0
Total % recovery:	irradiated	101.64 ± 1.34	95.77 ± 0.2	96.36 ± 1.54	96.54 ± 0.44	92.50 ± 3.21	91.92 ± 0.41	94.73 ± 0.6
	dark		97.56 ± 1.11	94.97 ± 1.35	97.84 ± 0.18	92.78 ± 0.99	87.94 ± 2.54	95.21 ± 2.22

Data obtained from Tables 3-5, pp. 36-38, Table 9, p. 42 of the study report. Means reported as calculated by the study author; standard deviations calculated by reviewer.

ND Not detected.

1 Unidentified radioactivity reported as minor unknowns (RT <33 min.) consisting of up to 6 compounds each comprising <4% of the applied radioactivity (Table 5, p. 38; Table 9, p. 42,; Figures 10-26, pp. 57-73).

2 Unidentified radioactivity reported as late eluting peaks (RT >39 min.) consisting of up to 15 compounds each comprising <4% of the applied radioactivity (Table 6, p. 39; Table 10, p. 43; Figures 10-26, pp. 57-73).

3 Determined to consist primarily of $^{14}CO_2$ (p. 25; Table 13, p. 46).

NA = Not analyzed.

C. TRANSFORMATION OF PARENT COMPOUND: Irradiation did not significantly affect the transformation of [C-phenyl-^{14}C]fenamidone on sandy loam soil; degradation was faster in the dark controls than in the irradiated samples (Figure 6, p. 54). In extracts from dark control soil, [^{14}C]fenamidone decreased from a mean 99.96 ± 1.30% (98.65-101.26%) of the applied at day 0 to 45.88 ± 0.82% (45.06-46.70%) at 5 days, 22.67 ± 0.88% (21.79-23.55%) at 15 days, and 10.59 ± 2.09% (8.50-12.68%) at 30 days (Table 9, p. 42). In extracts from irradiated soil, [^{14}C]fenamidone decreased to 49.03 ± 0.84% (48.18-49.87%) at 9 days and 26.01 ± 1.53% (24.48-27.54%) at 30 days (Table 5, p. 38). The study author suggested that the slower rate of transformation of [^{14}C]fenamidone on irradiated soil was due to difficulty in maintaining a constant soil moisture content (p. 30).

HALF-LIFE: The half-lives for fenamidone in the irradiated and dark control soil were determined by the reviewer to be 16.4 and 9.5 days, respectively, using linear regression analysis based on first-order kinetics as calculated by Corel Quattro Pro 8 software. DT50 and DT90 values were calculated by the study author using KIM, a nonlinear two-compartment Schering AG kinetic modeling program that determines time for test substance concentration to decline to 50% and 10% of initial concentration. Of the three models tested by the study author, KIM produced the best fit (>-0.995). The models used by the study author are compared in the Reviewer's Comments section of this DER.

Half-lives*

Test system	First order linear			DT50 (days)	DT90 (days)
	Half-life (days)	Regression equation	r^2		
Irradiated	16.4	Linear form y = mx + b as $lnC = -kt + lnC_0$; lnC_0 is initial concentration (b = y intercept), lnC is concentration at time t (y), k is the slope (m), t is time (x) or $kt = lnC_0 - lnC$. Half-life (t ½) = -(ln 2/k).	0.899	9.2	49.9
Dark	9.5		0.925	3.6	25.9

*Half-lives calculated by the reviewer using data obtained from Table 5, p. 38 and Table 9, p. 42 of study report. DT50 and DT90 values were calculated by the study author using KIM software (pp. 29-30, Tble 13, p. 46).

TRANSFORMATION PRODUCTS: The two major transformation products identified in irradiated and dark control soil extracts were RPA408056 and RPA717879. RPA408056 was detected at maximums of 20.41% (9 days) and 17.78% (30 days) of the applied radioactivity in the dark control and irradiated soil extracts, respectively, and RPA717879 was detected at maximums of 11.21% (21 days) and 12.44% (30 days), respectively (Table 5, p. 38; Table 9, p. 42). Minor transformation products in the soil extracts included RPA409445, RPA405862, RPA406012 and RPA410914 (dark soil extracts only), plus up to twenty-one unidentified [^{14}C]compounds each detected at <4% of the applied radioactivity (Tables 5-6, pp. 38-39; Tables 9-10, pp.42-43).

Table 8: Chemical names for identified transformation products of fenamidone

Applicant's Code Name	CAS Number	Chemical Names	Chemical formula	Molecular weight (g/mol)	Smiles string
RPA408056		IUPAC: 4-Methyl-2-methylthio-4-phenyl-2-imidazolin-5-one IUPAC: 5-Methyl-2-methylthio-5-phenyl-3,5-dihydroimidazol-4-one CAS: 4*H*-Imidazol-4-one, 3,5-dihydro-5-methyl-2-(methylthio)-5-phenyl-		220.3	
RPA405862	153969-11-0	IUPAC: 4-Methyl-4-phenyl-1-phenylaminoimidazolidin-2,5-dione IUPAC: 5-Methyl-5-phenyl-3-phenylaminoimidazolidine-2,4-dione CAS: 2,4-Imidazolidinedione, 5-methyl-5-phenyl-3-(phenylamino)-		281.3	
RPA717879	6843-49-8	IUPAC: 5-Methyl-5-phenylimidazolidine-2,4-dione CAS: 2,4-Imidazolidinedione, 5-methyl-5-phenyl-			
RPA409445		IUPAC: 3-(4-Aminophenylamino)-5-methyl-5-phenylimidazolidine-2,4-dione CAS: 2,4-Imidazolidinedione, 3-(4-aminophenylamino)-5-methyl-5-phenyl-			
RPA406012	151022-56-9	IUPAC: 5-Methyl-2-methylthio-3-(4-nitrophenylamino)-5-phenyl-3,5-dihydroimidazol-4-one CAS: 4*H*-Imidazol-4-one, 3,5-dihydro-5-methyl-2-(methylthio)-3-[(4-nitrophenyl)amino]-5-phenyl-			
RPA410914		IUPAC: 5-Methyl-2-methylthio-3-(2-nitrophenylamino)-5-phenyl-3,5-dihydroimidazol-4-one CAS: 4*H*-Imidazol-4-one, 3,5-dihydro-5-methyl-2-(methylthio)-3-(2-nitrophenylamino)-5-phenyl-			

Data obtained from pp. 48-50, 58-73 of the study report.

NONEXTRACTABLE AND EXTRACTABLE RESIDUES: Extractable [^{14}C]residues decreased from 101.15 ± 1.33% (99.82-102.49%) of the applied at day 0 to 65.34-73.34% at 21-30 days in dark control soil and 76.61-86.25% at 15-30 days in irradiated soil (Tables 3-4, pp. 36- 37). Nonextractable [^{14}C]residues in increased from 0.49 ± 0.0% (0.48-0.49%) of the applied at day 0 to 13.51-16.35% at 21-30 days in dark control soil and 8.36-11.47% at 15-30 days in irradiated soil; 2.67-4.14% of the applied was associated with the fulvic acid (in 9- and 30-day dark control and 15- and 30-day irradiated soil samples), 2.80-4.81% with humic acid and 4.64-7.40% with humins (Table 15, p. 47).

VOLATILIZATION: At 30 days posttreatment, volatilized $^{14}CO_2$ totaled 8.13 ± 2.0% (6.14-10.13%) of the applied for dark control soils and 2.25 ± 0.34% (1.90-2.59%) for irradiated soils; organic [^{14}C]volatiles were ≤0.03% of the applied (Tables 3-4, pp. 36-37; Table 14, p. 46).

TRANSFORMATION PATHWAY: A transformation pathway for the degradation of fenamidone on soil was proposed by the registrant; no unique transformation products resulted from irradiation (pp. 30, 100). Fenamidone degraded to 5-methyl-2-methylthio-5-phenyl-3,5-dihydroimidazol-4-one (RPA408056) via loss of the aniline ring, with further degradation to 5-methyl-5-phenylimidazolidine-2,4-dione (RPA717879) via hydrolysis to release the methylthio group and eventual mineralization to CO_2.

D. SUPPLEMENTARY EXPERIMENT-RESULTS: No supplementary experiments were performed.

III. STUDY DEFICIENCIES: No deficiencies were identified. This study, conducted with [C-phenyl-U-^{14}C]-labeled fenamidone, can be used to partially satisfy Subdivision N Guideline §161-3 data requirements. This study plus the soil photolysis study conducted with [N-phenyl-^{14}C]-labeled fenamidone (MRID 45385901) fully satisfy Subdivision N Guideline §161-3.

IV. REVIEWER'S COMMENTS

1. In the document *Reduced Risk Rationale for the Use of Fenamidone on Potatoes and Vegetables* (B0003264, no MRID), it is reported that fenamidone is the S-enantiomer compound with none of the R-enantiomer present (p. 16). It is further stated that analysis demonstrated that all of the metabolites of fenamidone that retain the imidazolinone ring are also pure S-enantiomers. No evidence was provided to support this statement.

   The registrant's code numbers used in this MRID do not match the code numbers presented in the *Reduced Risk Rationale*. For example, RPA405862 is RPA410193 and RPA408056 is RPA412708. The reason for the different code numbers was not discussed in any document, but the registrant does note in the *Reduced Risk Rationale* that the racemic mixtures were often

referenced in the original study reports. To avoid confusion, the chemical codes used in each study report are used throughout this DER.

2. Multiple IUPAC names were found for fenamidone and several of its transformation products. It could not be determined which name was currently preferred. All of the chemical names that were used in the MRIDs in this data package are included in the *Chemical names for identified transformation products* table in this DER and with the attached chemical structures.

3. The study author determined half-life/DT_{50} (50% decline time) values using first order least squares linear regression, Timme-Freshe square-root of first order decay, and KIM two compartment decay kinetic modeling analyses (pp. 46, 108-117). The results of these calculations are compared below:

Half-life/DT_{50} values of [C-phenyl-U-^{14}C]fenamidone on sandy loam soil:

Regression program		Half-life	r^2	DT_{50}	DT_{90}
First Order - linear least squares regression using Microsoft Excel 5. Linear form y = mx + b as lnC= -kt + lnC_0; lnC_0 is initial concentration (b = y intercept), lnC is concentration at time t (y), k is the slope (m), t is time (x) or kt = lnC_0 - lnC. Half-life (t ½) = -(ln 2/k). DT_{90} = -(ln 10/k).	irradiated	15.8 days	0.935	NA[1]	52.4 days
	dark	9.1 days	0.945	NA	30.4 days
Timme-Freshe - Bayer AG program (V.2.0) fits data to 1st, 1.5 and 2nd order decay curves. A square-root of the 1st order decline curve was utilized.	irradiated	7.0 days	0.989	NA	ND[2]
	dark	2.4 days	0.973	NA	26.5 days
KIM - Schering AG kinetic modeling program determines time for test substance concentration to decline to 50% and 10% of initial concentration.	irradiated	NA	NA	9.2 days	49.9 days
	dark	NA	NA	3.6 days	25.9 days

[1]NA = not applicable.
[2]ND = not determined.
Data obtained from pp. 46, 108-117 of the study report.

4. It appears that the registrant calculated the reported first order half-lives for fenamidone of 15.78 days (r^2 = 0.9349) and 9.14 (r^2 = 0.9445) days on irradiated and dark control soil, respectively, using mean values of fenamidone (percent of applied radioactivity) detected at each sampling interval (pp. 110, 111). It is preferred that individual replicate values are used for calculations to more accurately reflect the behavior of the compound. However, similar degradation half-lives for fenamidone (16.4 days, r^2 = 0.899 and 9.5 days, r^2 = 0.927 for irradiated and dark control soil, respectively) were determined by the Dynamac reviewer using [^{14}C]fenamidone concentrations at all sampling intervals and least squares linear regression analysis assuming degradation followed first order kinetics as calculated by Corel Quattro Pro 8 software.

5. Text and results indicate that each photolysis unit was individually attached to volatiles traps. Figure 3 (p. 51) implies the photolysis units were connected in series to the traps.

6. The registrant reported that recoveries of radioactivity applied to HPLC columns yielded an overall mean of >90%; however, no supporting data were provided (p. 25).

7. Soil samples were initially extracted the day of sampling (p. 35). Extracted soil samples and soil extracts were stored at unspecified temperatures until further extraction and/or analysis. Extractions were completed within 7 days of sampling and extracts were stored up to 88 days prior to HPLC analysis. The registrant reports that the HPLC and TLC profiles of parent fenamidone and transformation products imply that the [^{14}C]compounds were stable under storage (p. 26).

8. Representative HPLC and TLC chromatograms presented on pp. 55-81 indicated good separation of peaks.

9. The registrant reported that the target application rate was based on the cumulative field maximum application rate of 10 x 150 g a.i./ha which was equivalent a soil surface application of 0.0797 mg a.i./petri dish based on a dish diameter of 2.6 cm (p. 107). The treated soil dishes received 0.0776-0.0790 mg a.i./dish (p. 121). Based on soil sample weight of 4 g, the treated soils received 19.4-19.75 µg a.i./g soil (pp. 15, 16). In the *Reduced Risk Rationale for the Use of Fenamidone on Potatoes and Vegetables* (B0003264, no MRID), it is stated that the maximum proposed per season application rate is 1.07 lb a.i./A (equivalent to 0.54 mg a.i./kg or 0.60 kg a.i/ha). The application rate was sufficient to allow for identification of all significant degradates.

V. REFERENCES: The following references were cited in the study:

1. Burr, C.M. In progress. [^{14}C]-RPA 407213 investigation of the chirality of RPA 407231 and major metabolites in animals, plants, soils and water. Rhône-Poulenc Document 210763.

2. Burr, C.M. and M.B. Simmonds. 1999. [^{14}C]-RPA 407213 route of degradation. Rhône-Poulenc Document 201609.

3. Walter, H., H.F. Frehse and Timme. 1993. *Pflazenschutz-Nachrichten Bayer*, Vol. 46, 3, pp. 265-288.

Figure 6: Composition of Extractable Radioactivity - Irradiated Soil

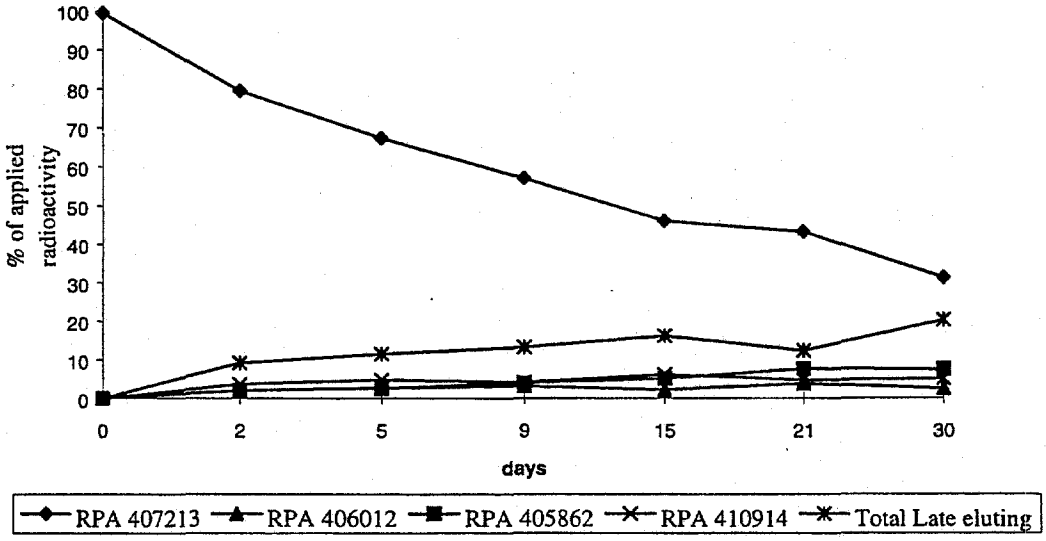


Figure 7: Composition of Extractable Radioactivity - Non-Irradiated Soil

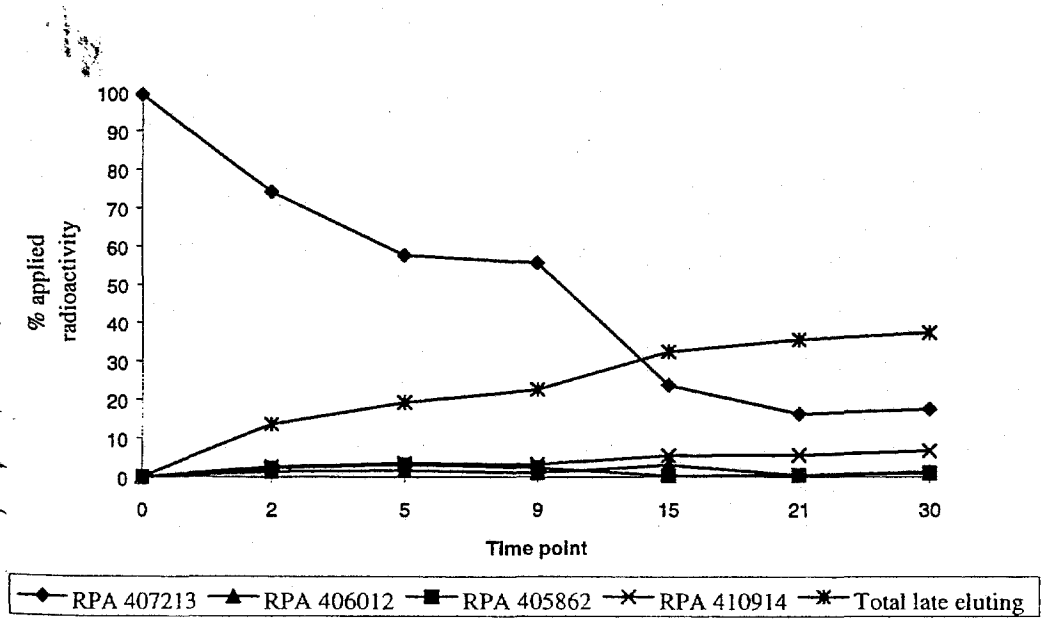


Figure 4: Distribution of Total Radioactivity for Irradiated Soil

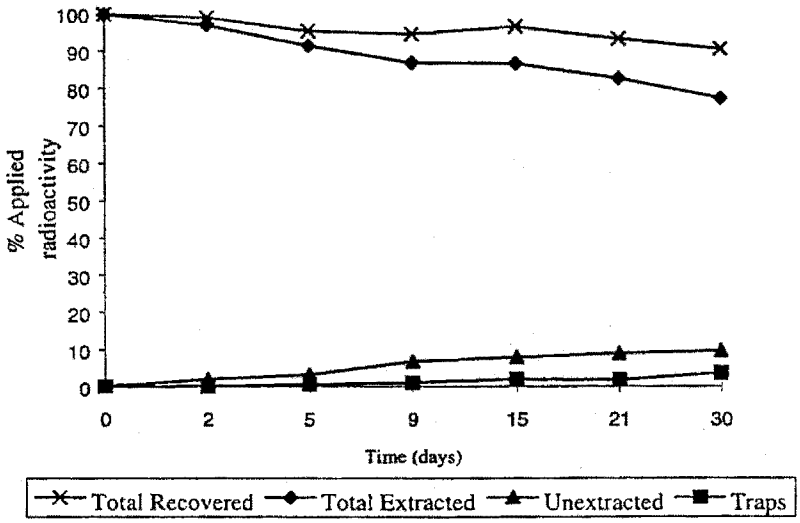


Figure 5: Distribution of Total Radioactivity for Non-Irradiated Soil

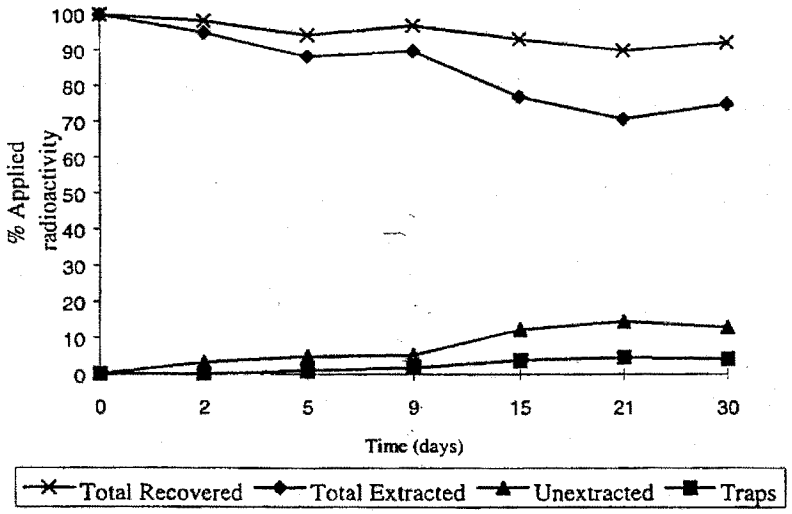


Figure 3: Diagram of Sample Dish Positions for Irradiated Samples

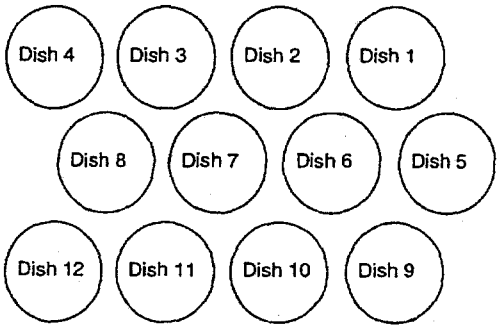


Figure 1: Diagram of Photolysis chamber

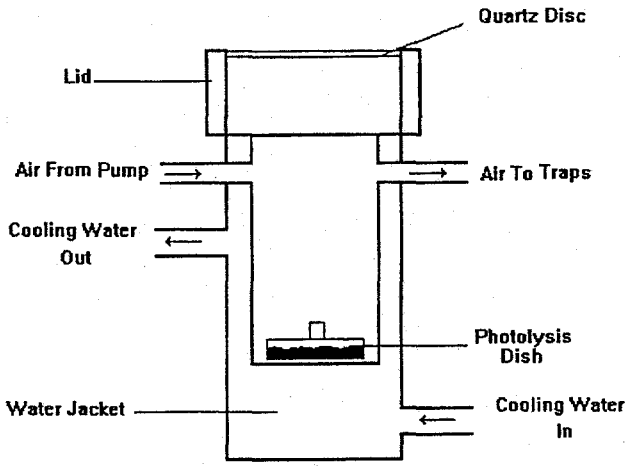


Figure 2: Schematic Diagram of Experimental Set-Up

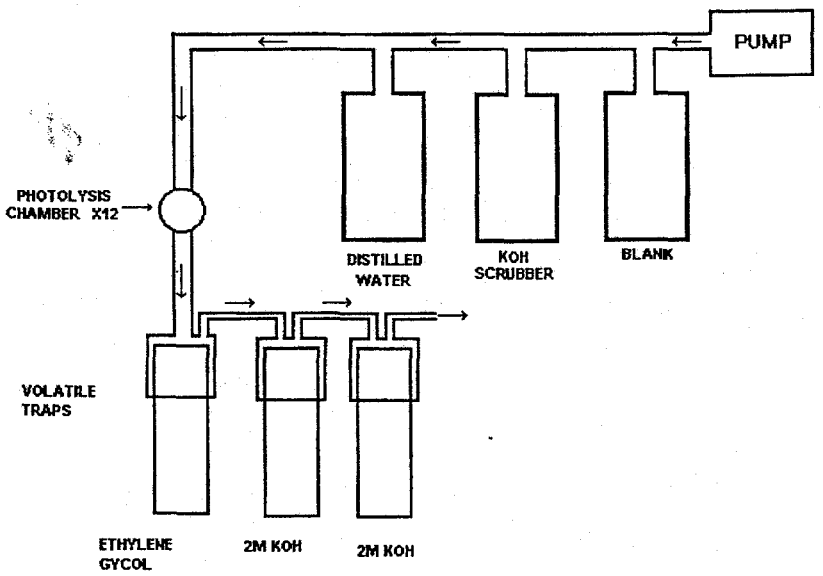


Table 13: DT_{50} and DT_{90} Values for RPA 407213

	Program	DT_{50} (days)	DT_{90} (days)	Fit*
Irradiated Soil	First Order (Excel)	19.64	65.25	0.9481
	KIM Power Rate	12.63	not calculated	-0.99866
	Timme-Frehse Sqrt 1" order	11.14	not calculated	0.9871
Non-Irradiated Soil	First Order (Excel)	10.96	36.40	0.8872
	KIM Two compartment decay	7.90	40.77	-0.98555
	Timme-Frehse 1.5" order	8.23	not calculated	0.9445

* These figures are all a measure of the "goodness of fit", but are not equivalent from analysis to analysis. In all cases, a value of ±1.000 would represent a perfect fit to the experimental data.

Table 14: Distribution of Unextracted Residues between Fulvic/Humic/Humin Fractions.

Dish	% Applied radioactivity unextracted	Soil Fraction	% of unextracted radioactivity in fraction	% of applied radioactivity in fraction
22 (15 day dark control)	12.19	0.01M Calcium chloride extract	0.96	0.11
		Fulvic acid	17.03	2.02
		Humic Acid	44.41	5.26
		Humin	37.60	4.46
18 (30 day dark control)	13.85	0.01M Calcium chloride extract	0.99	0.13
		Fulvic acid	19.17	2.55
		Humic Acid	39.02	5.18
		Humin	40.81	5.42
5 (21 day irradiated)	8.89	0.01M Calcium chloride extract	1.16	0.10
		Fulvic acid	21.38	1.84
		Humic Acid	42.24	3.63
		Humin	35.22	3.03
2 (30 day irradiated)	9.63	0.01M Calcium chloride extract	1.39	0.13
		Fulvic acid	21.74	1.99
		Humic Acid	34.90	3.20
		Humin	41.97	3.84

Table 15: Typical RRT and R_f of Certified Reference Standards

	RRT * (HPLC method 1)	Rf*			RRT (HPLC method 1)	Rf
RPA 407213	1.00	0.56		RPA 408056	0.33	0.34
RPA 410914	1.10	0.56		RPA 717879	0.30	0.24
RPA 405862	0.57	0.33		RPA 406012	1.07	0.47

* calculated from separate analysis of standards

Table 11: Concentration of Metabolites for Non-Irradiated Soil

		Concentration (μg g^{-1})					
Time Point	Dish no.	RPA 407213	RPA 405862	RPA 406012	RPA 410914	Total unknowns (RRT < 1.0)	Total Late eluting (RRT > 1.2)
RRT		1.00	0.58	1.07	1.12	<1.00	>1.2
Zero time	25	19.94	n.d.	n.d.	n.d.	n.d.	n.d.
	26	19.65	n.d.	n.d.	n.d.	n.d.	n.d.
		19.80					
2 days	13	14.89	0.45	0.30	0.44	0.21	2.63
	24	14.60	0.41	0.21	0.59	0.24	2.74
Mean		**14.75**	**0.43**	**0.26**	**0.52**	**0.22**	**2.68**
5 days	16	11.86	0.78	0.32	0.73	0.18	3.63
	21	11.07	0.41	0.36	0.70	0.40	4.01
Mean		**11.46**	**0.60**	**0.34**	**0.72**	**0.29**	**3.82**
9 days	15B*	12.89	0.06	n.d.	0.58	0.35	4.34
	23	9.22	0.84	0.40	0.68	1.03	4.63
Mean		**11.06**	**0.45**	**0.20**	**0.63**	**0.69**	**4.48**
15 days	22	4.71	0.06	0.60	1.09	1.57	6.46
Mean		**4.71**	**0.06**	**0.60**	**1.09**	**1.57**	**6.46**
21 days	17	2.76	0.14	0.00	1.27	1.79	7.10
	20	3.70	0.03	0.19	0.96	1.22	7.03
Mean		**3.23**	**0.08**	**0.10**	**1.11**	**1.51**	**7.06**
30 days	18	2.90	0.34	0.50	0.87	2.10	7.38
	19	4.05	0.06	n.d.	1.79	1.06	7.47
Mean		**3.48**	**0.20**	**0.25**	**1.33**	**1.58**	**7.42**

Table 12: Metabolite Profile for Non-Irradiated Soil: TLC Analysis

		Presence of Component				
Time Point	Dish no.	RPA 407213	RPA 405862	RPA 406012	origin	Other unknowns
0	25	x	x		x	
2 Day	13	x	x	x	x	x
5 day	16	x	x	x	x	x
9 day	23	x	x	x	x	x
15 days	22	x	x	x	x	x
21 days	17	x	x	x	x	x
30 days	18	x	x	x	x	x

x - presence of component

Table 10: Profile of Late Eluting Peaks for Non-Irradiated Soil: HPLC Analysis

		% Applied Radioactivity											
Time Point	Dish No:	HH1	HH2	HH3	HH4	HH5	HH6	HH7	HH8	HH9	HH10	HH11	HH12
RRT		1.32	1.40	1.58	1.63	1.73	1.75	1.81	1.98	2.00	2.16	2.21	2.33
2 days	13	n.d.	1.81	0.76	n.d.	n.d.	2.31	n.d.	n.d.	3.18	0.72	1.37	1.77
	24	0.86	1.05	0.52	n.d.	0.48	2.81	n.d.	n.d.	2.86	n.d.	2.48	2.05
Mean		**0.43**	**1.43**	**0.64**		**0.24**	**2.56**			**3.02**	**0.36**	**1.92**	**1.91**
5 days	16	n.d.	2.00	1.76	n.d.	n.d.	2.76	n.d.	n.d.	3.85	n.d.	n.d.	3.81
	21	n.d.	1.78	0.90	n.d.	n.d.	2.61	n.d.	2.16	2.88	n.d.	n.d.	3.73
Mean			**1.89**	**1.33**			**2.69**		**1.08**	**3.36**			**3.77**
9 days	15B*	n.d.	1.70	1.16	n.d.	1.36	n.d.	2.96	1.89	3.92	0.53	0.92	1.55
	23	n.d.	2.37	1.85	n.d.	n.d.	n.d.	3.18	n.d.	5.31	n.d.	n.d.	3.32
Mean			**2.37**	**1.85**		**n.d.**		**3.18**	**n.d.**	**5.31**	**n.d.**	**n.d.**	**3.32**
15 days	22	n.d.	1.23	n.d.	n.d.	3.23	4.51	n.d.	1.33	4.51	0.90	3.61	2.99
Mean			**1.23**	**n.d.**	**n.d.**	**3.23**	**4.51**	**n.d.**	**1.33**	**4.51**	**0.90**	**3.61**	**2.99**
21 days	17	n.d.	n.d.	4.20	n.d.	1.51	2.87	n.d.	n.d.	6.53	1.05	4.08	4.47
	20	1.82	n.d.	1.82	0.75	n.d.	5.64	n.d.	n.d.	6.17	0.78	5.99	3.43
Mean		**0.91**		**3.01**	**0.37**	**0.76**	**4.26**			**6.35**	**0.92**	**5.04**	**3.95**
30 days	18	1.09	n.d.	1.47	n.d.	1.04	4.75	n.d.	n.d.	5.09	1.19	4.85	3.23
	19	n.d.	1.83	n.d.	n.d.	n.d.	7.04	n.d.	n.d.	5.77	1.62	6.25	5.81
Mean		**0.55**	**0.92**	**0.74**		**0.52**	**5.90**			**5.43**	**1.40**	**5.55**	**4.52**

		% Applied Radioactivity												
Time Point	Dish No:	HH13	HH14	HH15	HH16	HH17	HH18	HH19	HH20	HH21	HH22	HH23	HH24	Total Late eluting
RRT		2.43	2.52	2.73	2.83	2.90	3.05	3.13	3.18	3.32	3.50	3.69	3.91	
2 days	13	n.d.	n.d.	n.d.	0.51	0.76	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	13.18
	24	n.d.	0.67	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	13.77
Mean			**0.33**		**0.25**	**0.38**								**13.47**
5 days	16	1.88	n.d.	0.92	n.d.	1.24	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	18.23
	21	3.69	n.d.	n.d.	n.d.	2.39	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	20.14
Mean		**2.79**		**0.46**	**n.d.**	**1.81**								**19.19**
9 days	15B	2.67	1.11	n.d.	n.d.	1.74	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	21.77
	23	2.57	0.97	n.d.	1.18	n.d.	0.54	0.86	n.d.	0.33	n.d.	0.37	n.d.	23.23
Mean		**2.57**	**0.97**		**1.18**	**n.d.**	**0.54**	**0.86**		**0.33**		**0.37**		**22.50**
15 days	22	1.76	n.d.	n.d.	2.75	n.d.	n.d.	0.81	1.33	0.62	1.23	n.d.	1.14	32.41
Mean		**1.76**	**n.d.**		**2.75**	**n.d.**	**n.d.**	**0.81**	**1.33**	**0.62**	**1.23**	**n.d.**	**1.14**	**32.41**
21 days	17	2.25	0.66	1.36	3.69	n.d.	n.d.	n.d.	2.95	n.d.	n.d.	n.d.	n.d.	35.62
	20	n.d.	1.82	0.54	1.32	0.78	n.d.	n.d.	3.03	n.d.	1.39	n.d.	n.d.	35.28
Mean		**1.13**	**1.24**	**0.95**	**2.51**	**0.39**			**2.99**		**0.70**			**35.45**
30 days	18	2.52	0.38	1.00	2.43	1.14	n.d.	1.04	2.00	1.09	1.28	0.76	0.67	37.03
	19	n.d.	0.74	1.31	n.d.	2.49	n.d.	n.d.	2.19	n.d.	1.71	n.d.	n.d.	37.49
Mean		**1.26**	**0.56**	**1.16**	**1.21**	**1.82**		**0.52**	**2.09**	**0.55**	**1.50**	**0.38**	**0.33**	**37.26**

Table 9: Metabolite Profile for Non-Irradiated Soil: HPLC Analysis

		% Applied Radioactivity						
Time Point	Dish no.	% in extract	RPA 407213	RPA 405862	RPA 406012	RPA 410914	Total unknowns* (RRT < 1.0)	Total Late eluting** (RRT > 1.2)
RRT			1.00	0.58	1.07	1.12	<1.00	>1.2
Zero time	25	100.09	100.09	n.d.	n.d.	n.d.	n.d.	n.d.
	26	98.62	98.62	n.d.	n.d.	n.d.	n.d.	n.d.
		99.36	**99.36**					
2 days	13	94.97	74.74	2.25	1.52	2.23	1.04	13.18
	24	94.33	73.28	2.08	1.06	2.95	1.19	13.77
Mean		**94.65**	**74.01**	**2.17**	**1.29**	**2.59**	**1.11**	**13.47**
5 days	16	87.87	59.53	3.91	1.62	3.68	0.90	18.23
	21	85.05	55.55	2.08	1.78	3.50	2.00	20.14
Mean		**86.46**	**57.54**	**3.00**	**1.70**	**3.59**	**1.45**	**19.19**
9 days	15B	91.41	64.68	0.30	n.d.	2.92	1.74	21.77
	23	84.35	46.30	4.22	2.02	3.43	5.15	23.23
Mean		**87.88**	**55.49**	**2.26**	**1.01**	**3.17**	**3.45**	**22.50**
15 days	22	72.70	23.63	0.29	3.03	5.47	7.87	32.41
Mean		**72.70**	**23.63**	**0.29**	**3.03**	**5.47**	**7.87**	**32.41**
21 days	17	65.53	13.83	0.71	n.d.	6.38	8.99	35.62
	20	65.87	18.59	0.15	0.94	4.79	6.12	35.28
Mean		**65.70**	**16.21**	**0.43**	**0.47**	**5.59**	**7.56**	**35.45**
30 days	18	70.72	14.58	1.69	2.51	4.38	10.53	37.03
	19	72.41	20.31	0.31	n.d.	8.96	5.33	37.49
Mean		**71.56**	**17.44**	**1.00**	**1.26**	**6.67**	**7.93**	**37.26**

* up to 5 components each <5%

** see table 10 for details of components

Table 7: Concentration of Metabolites for Irradiated Soil

		Concentration (µg g^{-1})					
Time Point	Dish no.	RPA 407213	RPA 405862	RPA 406012	RPA 410914	Total unknowns (RRT < 1.0)	Total Late eluting (RRT > 1.2)
RRT		1.00	0.58	1.07	1.12	<1.0	RRT= >1.2
0	25	19.94	n.d.	n.d.	n.d.	n.d.	n.d.
	26	19.65	n.d.	n.d.	n.d.	n.d.	n.d.
Mean		**19.80**					
2 Day	1	16.05	0.42	0.38	0.62	0.18	1.81
	12	15.56	0.39	0.39	0.83	0.14	1.80
Mean		**15.80**	**0.40**	**0.38**	**0.73**	**0.16**	**1.81**
5 day	4	13.07	0.46	0.58	0.95	0.23	2.27
	9	13.69	0.55	0.47	0.92	0.44	2.29
Mean		**13.38**	**0.50**	**0.53**	**0.94**	**0.33**	**2.28**
9 day	6A	12.07	0.92	0.59	0.76	0.51	2.59
	11	10.58	0.63	0.64	0.89	0.95	2.66
Mean		**11.33**	**0.78**	**0.62**	**0.83**	**0.73**	**2.62**
15 days	3	9.41	0.88	n.d.	1.39	1.35	3.21
	10	8.87	1.13	0.86	1.05	1.65	3.20
Mean		**9.14**	**1.01**	**0.43**	**1.22**	**1.50**	**3.21**
21 days	5	8.56	1.51	0.75	0.93	1.56	2.44
Mean		**8.56**	**1.51**	**0.75**	**0.93**	**1.56**	**2.44**
30 days	2	6.19	1.48	0.49	1.00	1.45	4.02
Mean		**6.19**	**1.48**	**0.49**	**1.00**	**1.45**	**4.02**

RRT = relative retention time (see Appendix 4)

Table 8: Metabolite Profile for Irradiated Soil: TLC Analysis

		Presence of Component				
Time Point	Dish no.	RPA 407213	RPA 405862	RPA 406012	origin	Other unknowns
0	25	X				
2 Day	1	X	X	X	X	
5 day	4	X		X	X	X
9 day	11	X	X	X	X	
15 days	3	X	X	X	X	X
21 days	5	X	X	X	X	X
30 days	2	X		X	X	X

X - presence of component

Table 6: Profile of Late Eluting Peaks - Irradiated Soil: HPLC Analysis

		% Applied Radioactivity									
Time Point	Dish No:	HH1	HH2	HH3	HH4	HH5	HH6	HH7	HH8	HH9	HH10
RRT		1.32	1.40	1.58	1.63	1.75	1.81	1.98	2.00	2.16	2.21
2 days	1	n.d.	1.64	n.d.	n.d.	n.d.	4.05	n.d.	3.41	n.d.	n.d.
	12	n.d.	2.35	n.d.	n.d.	n.d.	2.07	n.d.	3.39	n.d.	n.d.
Mean			**2.00**				**3.06**		**3.40**		
5 days	4	n.d.	2.31	n.d.	n.d.	n.d.	5.78	n.d.	3.31	n.d.	n.d.
	9	n.d.	2.30	n.d.	n.d.	n.d.	3.52	n.d.	3.65	n.d.	n.d.
Mean			**2.31**				**4.65**		**3.48**		
9 days	6A	n.d.	2.28	0.95	n.d.	n.d.	2.69	0.81	2.50	n.d.	n.d.
	11	n.d.	1.16	1.25	n.d.	3.11	n.d.	n.d.	n.d.	2.94	n.d.
Mean			**1.72**	**1.10**		**1.56**	**1.34**	**0.40**	**1.25**	**1.47**	
15 days	3	n.d.	n.d.	1.11	n.d.	1.47	2.58	1.74	2.05	n.d.	1.95
	10	n.d.	n.d.	2.53	1.67	n.d.	3.84	n.d.	4.49	n.d.	n.d.
Mean				**1.82**	**0.83**	**0.74**	**3.21**	**0.87**	**3.27**		**0.97**
21 days	5	n.d.	1.02	1.08	n.d.	2.24	0.34	n.d.	2.58	0.55	1.23
Mean			**1.02**	**1.08**		**2.24**	**0.34**		**2.58**	**0.55**	**1.23**
30 days	2	n.d.	2.21	2.64	n.d.	n.d.	4.10	n.d.	3.12	1.50	1.77
Mean			**2.21**	**2.64**			**4.10**		**3.12**	**1.50**	**1.77**

		% Applied Radioactivity									
Time Point	Dish No:	HH11	HH12	HH13	HH14	HH15	HH16	HH17	HH18	HH19	Total Late eluting
RRT		2.33	2.43	2.52	2.83	2.90	3.18	3.32	3.50	3.91	
2 days	1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	9.10
	12	n.d.	1.22	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	9.04
Mean			**0.61**								**9.07**
5 days	4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	11.40
	9	2.03	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	11.51
Mean		**1.02**									**11.46**
9 days	6A	1.45	0.83	0.72	n.d.	0.77	n.d.	n.d.	n.d.	n.d.	12.99
	11	3.96	n.d.	n.d.	n.d.	0.93	n.d.	n.d.	n.d.	n.d.	13.34
Mean		**2.70**	**0.41**	**0.36**		**0.85**					**13.17**
15 days	3	2.95	n.d.	n.d.	1.53	n.d.	n.d.	n.d.	n.d.	n.d.	16.10
	10	2.82	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	16.08
Mean		**2.89**			**0.76**						**16.09**
21 days	5	1.26	n.d.	n.d.	0.95	n.d.	0.40	n.d.	n.d.	n.d.	12.27
Mean		**1.26**			**0.95**		**0.40**				**12.27**
30 days	2	2.44	n.d.	n.d.	1.46	n.d.	n.d.	n.d.	n.d.	n.d.	20.16
Mean		**2.44**			**1.46**						**20.16**

Table 5: Metabolite Profile for Irradiated Soil: HPLC Analysis

		% Applied Radioactivity						
Time Point	Dish no.	% in extract	RPA 407213	RPA 405862	RPA 406012	RPA 410914	Total unknowns * (RRT < 1.0)	Total Late eluting** (RRT > 1.2)
RRT			1.00	0.58	1.07	1.12	<1.00	>1.2
0	25	100.09	100.09	n.d.	n.d.	n.d.	n.d.	n.d.
	26	98.62	98.62	n.d.	n.d.	n.d.	n.d.	n.d.
Mean		**99.36**	**99.36**					
2 Day	1	95.94	80.53	2.10	1.89	3.10	0.90	9.10
	12	94.29	78.10	1.95	1.95	4.18	0.71	9.04
Mean		**95.11**	**79.32**	**2.02**	**1.92**	**3.64**	**0.80**	**9.07**
5 day	4	86.19	65.59	2.30	2.92	4.76	1.14	11.40
	9	89.55	68.70	2.77	2.36	4.64	2.19	11.51
Mean		**87.87**	**67.15**	**2.53**	**2.64**	**4.70**	**1.66**	**11.46**
9 day	6A	83.59	60.60	4.64	2.98	3.83	2.58	12.99
	11	78.50	53.08	3.18	3.21	4.46	4.76	13.34
Mean		**81.04**	**56.84**	**3.91**	**3.10**	**4.14**	**3.67**	**13.17**
15 days	3	75.79	47.23	4.43	n.d.	6.96	6.80	16.10
	10	79.85	44.49	5.68	4.31	5.25	8.27	16.08
Mean		**77.82**	**45.86**	**5.05**	**2.15**	**6.11**	**7.54**	**16.09**
21 days	5	72.05	42.94	7.60	3.74	4.67	7.82	12.27
Mean		**72.05**	**42.94**	**7.60**	**3.74**	**4.67**	**7.82**	**12.27**
30 days	2	65.30	31.08	7.42	2.46	5.04	7.30	20.16
Mean		**65.30**	**31.08**	**7.42**	**2.46**	**5.04**	**7.30**	**20.16**

*Up to 9 components each < 5%

** for detailed profile see Table 6.

Table 4: Total Recovery and Distribution of Radioactivity in Non-Irradiated Soil

		% Applied Radioactivity						
		Extract						
Time Point	Dish No:	1	2	3	Total Extracted	Volatile	Unextracted	Total recovery
0 hours	25	100.09	0.24	N/A	100.33	N/A	0.09	100.42
	26	98.62	0.26	N/A	98.88	N/A	0.10	98.98
Mean		**99.36**	**0.25**	**N/A**	**99.60**	**N/A**	**0.10**	**99.70**
2 days	13	92.81	2.16	N/A	94.97	0.49	2.97	98.43
	24	92.12	2.21	N/A	94.34	0.41	3.55	98.30
Mean		**92.47**	**2.19**	**N/A**	**94.65**	**0.45**	**3.26**	**98.37**
5 days	16	85.24	2.63	1.26	89.13	0.73	4.51	94.37
	21	82.02	3.03	1.91	86.97	1.22	5.27	93.46
Mean		**83.63**	**2.83**	**1.59**	**88.05**	**0.98**	**4.89**	**93.92**
9 days	15B	88.00	3.41	1.52	92.93	2.05	5.08	100.06
	23	79.72	4.63	1.91	86.26	1.42	5.44	93.12
Mean		**83.86**	**4.02**	**1.71**	**89.60**	**1.73**	**5.26**	**96.59**
15 days	14A*	80.50	*	2.25	82.75	2.00	6.18	90.93
	22	66.73	5.97	4.09	76.79	3.83	12.19	92.81
Mean		**66.73**	**5.97**	**4.09**	**76.79**	**3.83**	**12.19**	**92.81**
21 days	17	59.14	6.39	4.37	69.90	4.52	13.41	87.84
	20	62.42	3.45	5.40	71.27	4.58	15.57	91.42
Mean		**60.78**	**4.92**	**4.89**	**70.59**	**4.55**	**14.49**	**89.63**
30 days	18	62.16	7.44	4.03	73.64	4.87	14.19	92.69
	19	65.63	6.78	3.59	76.00	3.48	11.93	91.41
Mean		**63.89**	**7.11**	**3.81**	**74.82**	**4.18**	**13.06**	**92.05**
							Overall mean recovery	**94.87**

N/A - not applicable

* dish excluded due to loss of extract 2 during processing

Table 3: Total Recovery and Distribution of Radioactivity in Irradiated Soil

		% Applied Radioactivity						
		Extract						
Time Point	Dish No:	1	2	3	Total Extracted	Volatile	Unextracted	Total recovery
0 hours	25	100.09	0.24	N/A	100.33	N/A	0.09	100.42
	26	98.62	0.26	N/A	98.88	N/A	0.10	98.98
Mean		**99.36**	**0.25**	**N/A**	**99.60**	**N/A**	**0.10**	**99.70**
2 days	1	95.94	1.68	N/A	97.62	0.06	1.85	99.52
	12	94.29	1.64	N/A	95.93	0.09	2.01	98.03
Mean		**95.11**	**1.66**	**N/A**	**96.77**	**0.0**	**1.93**	**98.78**
5 days	4	86.19	2.15	1.06	89.40	0.28	2.85	92.52
	9	89.55	2.69	1.09	93.34	0.98	3.63	97.94
Mean		**87.87**	**2.42**	**1.08**	**91.37**	**0.63**	**3.24**	**95.23**
9 days	6A	83.59	4.03	1.43	89.05	1.01	7.08	97.13
	11	78.50	3.54	2.24	84.27	1.01	6.35	91.64
Mean		**81.04**	**3.78**	**1.83**	**86.66**	**1.01**	**6.72**	**94.39**
15 days	3	75.79	6.05	2.42	84.26	1.90	8.12	94.28
	10	79.85	6.09	2.61	88.55	1.97	7.67	98.19
Mean		**77.82**	**6.07**	**2.52**	**86.41**	**1.93**	**7.90**	**96.24**
21 days	5	72.05	7.23	3.09	82.37	1.73	8.89	92.98
	8*	63.07	6.24	2.57	71.87	2.07	7.45	81.40
Mean		**72.05**	**7.23**	**3.09**	**82.37**	**1.73**	**8.89**	**92.98**
30 days	2	65.30	8.49	3.24	77.03	3.56	9.63	90.22
	7*	54.29	7.03	2.66	63.98	4.24	10.99	79.20
Mean		**65.30**	**8.49**	**3.24**	**77.03**	**3.56**	**9.63**	**90.22**
							Overall mean recovery	**95.99**

N/A - not applicable

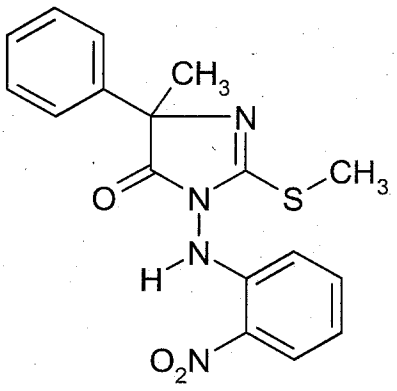
* dishes excluded due to very low overall recovery

RPA 410914

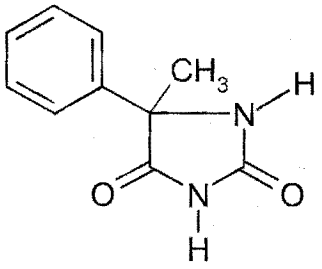
IUPAC name: 5-Methyl-2-methylthio-3-(2-nitrophenylamino)-5-phenyl-3,5-dihydroimidazol-4-one

CAS name: 4*H*-Imidazol-4-one, 3,5-dihydro-5-methyl-2-(methylthio)-3-(2-nitrophenylamino)-5-phenyl-

CAS #: N/A



RPA 717879
IUPAC name: 5-Methyl-5-phenylimidazolidine-2,4-dione
CAS name: 2,4-Imidazolidinedione, 5-methyl-5-phenyl-
CAS #: 6843-49-8



Attachment 3

Illustration of Test System
Artificial Light Irradiation Spectrum

Figure 1: Diagram of Photolysis chamber

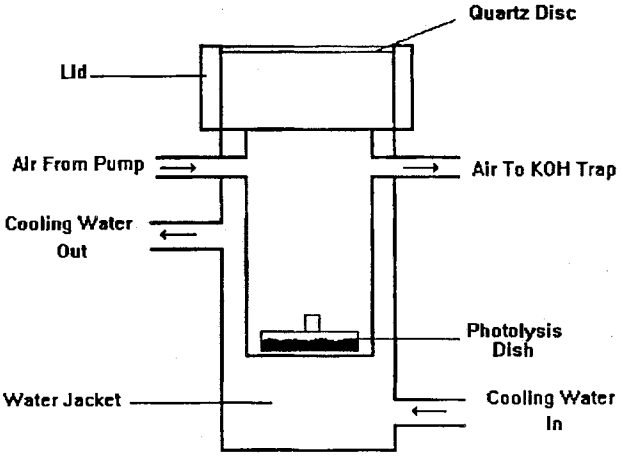
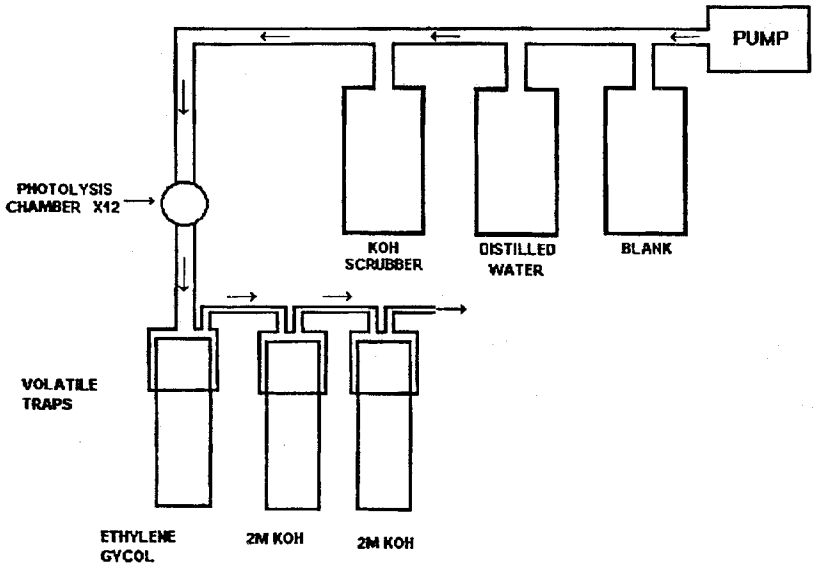


Figure 2: Schematic Diagram of Experimental Set-Up



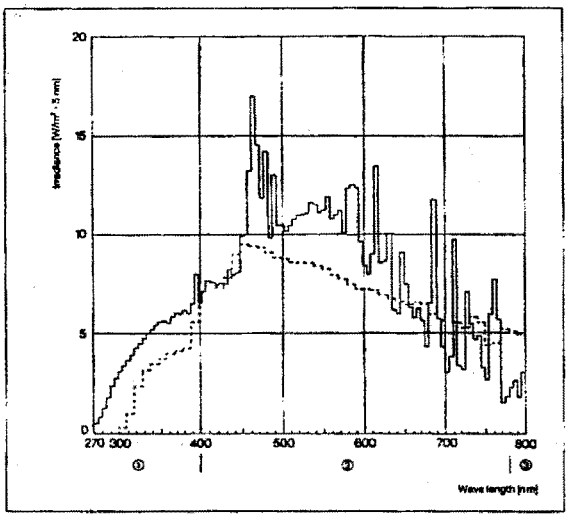
Appendix 2: Light Source

2.1: Spectral Energy Distribution

The Hanau Suntest is fitted with an artificial xenon light source with UV filters which subjects the samples to radiation of wavelength 290 - 800+ nm. The spectral energy distribution of the xenon source in the Hanau Suntest machine is shown below.

—— SUNTEST radiation without additional UV filter

----- Global radiation according to daylight D65



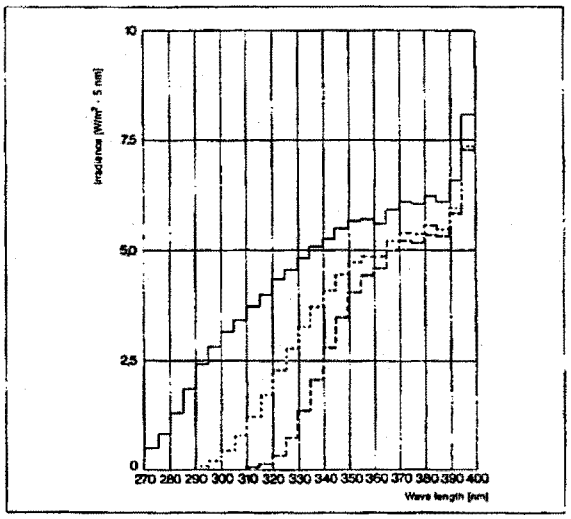
① UV-Radiation
② visible light
③ IR-Radiation

—— without additional filters

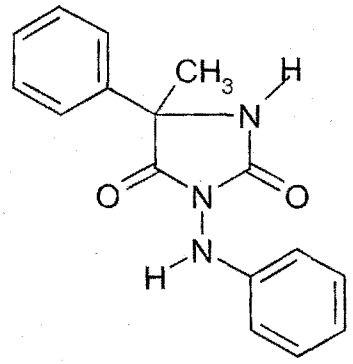
----- with additional filters of special UV filterglass

---- with additional windowglass filters for test procedures according to simulation of sunlight behind glass.

All figures refer to the maximum irradiance level.



RPA 405862
IUPAC name: 5-Methyl-5-phenyl-3-phenylaminoimidazolidine-2,4-dione
CAS name: 2,4-Imidazolidinedione, 5-methyl-5-phenyl-3-(phenylamino)-
CAS #: 153969-11-0

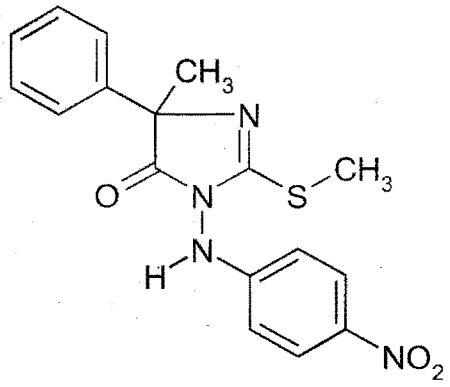


RPA 406012

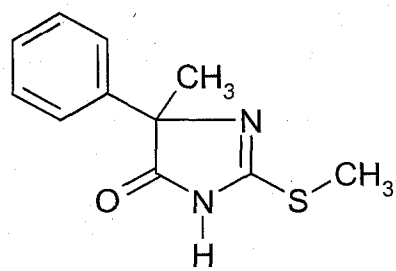
IUPAC name: 5-Methyl-2-methylthio-3-(4-nitrophenylamino)-5-phenyl-3,5-dihydroimidazol-4-one

CAS name: 4*H*-Imidazol-4-one, 3,5-dihydro-5-methyl-2-(methylthio)-3-[(4-nitrophenyl)amino]-5-phenyl-

CAS #: 151022-56-9



RPA 408056
IUPAC name: 5-Methyl-2-methylthio-5-phenyl-3,5-dihydroimidazol-4-one
CAS name: 4*H*-Imidazol-4-one, 3,5-dihydro-5-methyl-2-(methylthio)-5-phenyl-
CAS #: N/A



RPA 409445

IUPAC name: 3-(4-Aminophenylamino)-5-methyl-5-phenylimidazolidine-2,4-dione

CAS name: 2,4-Imidazolidinedione, 3-(4-aminophenylamino)-5-methyl-5-phenyl-

CAS #: N/A